IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ian H. FRAZER	)	Confirmation No: 8451
	)	
Application No.: 10/534,130	)	Art Unit: 1633
	)	
Filed: December 30, 2005	)	Examiner: Janet Epps Ford

For: A METHOD FOR OPTIMISING GENE EXPRESSION USING SYNONYMOUS CODON OPTIMISATION

Commissioner for Patents
P.O. Box 1450, **Mail Stop Amendment**
Alexandria, VA 22313-1450

Declaration under 37 C.F.R. § 1.132

I, Ian H. Frazer, declare and say:

1. I received Bachelor of Science, Bachelor of Medicine and Bachelor of Surgery degrees from the University of Edinburgh, Scotland, and my MD degree from the University of Melbourne. I am the Director of the Diamantina Institute for Cancer, Immunology and Metabolic Medicine at the University of Queensland. A copy of my *Curriculum Vitae* is attached to this declaration as APPENDIX A.

2. I am the inventor of the captioned application.

3. An immune response in an animal can be induced by delivering a gene to that animal, whereby expression of the gene results in an immune response to the encoded polypeptide. Prior workers had developed tables of preferred codons for optimizing gene expression in isolated cells. However, when a gene is delivered to a whole animal to generate an immune response, it is not known what cells are important for expression of the gene in order to optimize the immune response in a desired way. Accordingly, the tables of preferred codons are not useful for optimizing the immune response in a whole animal.

4. The present invention therefore is directed to methods of making synthetic polynucleotides that encode polypeptides that produce an immune response and to methods of modulating an immune response to a polypeptide encoded by a polynucleotide by changing the polynucleotide sequence.

5. I describe below experiments and data generated using the methods of the invention. These experiments demonstrate that the claimed methods can be used to prepare polynucleotides that produce an enhanced immune response when delivered to a whole animal, and to modulate that immune response.

6. Thus, a series of expression constructs was prepared encoding the human papillomavirus E7 protein in which the codons for individual amino acids were varied. For example, in one construct, the codon GCG was used to encode each of the alanine residues in the polypeptide, while in other constructs the alanines were encoded by GCA, GCC and GCT respectively. The set of constructs used is shown in the table set forth in APPENDIX B.

7. The constructs were prepared in the pCDNA3 vector (Invitrogen) and were delivered to mice using the Helios Gene Gun System (Biorad). The immune response in the mice was measured in an ELISA assay. The results obtained were used to generate a table of immune response preferences for codons, as shown in APPENDIX C.

8. This table was then used to design three optimized constructs (O1 to O3) for the E7 protein and an E7-derived peptide (peptide 101) that were delivered to mice as described above, and the corresponding immune response (antibody response and cellular response) measured. The results are shown in APPENDIX D. The optimized sequences gave rise to significantly larger antibody responses than the wild-type construct. A de-optimized construct, W, gave a very low antibody response, appearing slightly lower but not statistically different from the wild-type (wt) codon usage (CU) construct.

9. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date: 7/11/2008

Ian Frazer